

with luminol to produce chemiluminescence in proportion to the initial glucose concentration. A limit of detection of 8×10^{-9} M peroxide was obtained with this system. Williams et al. (1976), Evaluation Of Peroxyoxalate Chemiluminescence For Determination Of Enzyme Generated Peroxide. *Anal. Chem.*, 48, 7 1003-1006 in a similar reaction concluded the limit of sensitivity of the peroxyoxalate system is an order of magnitude poorer than that of the luminol system.

Therefore, until now the oxalic ester system (oxalate system) was generally thought to have little utility for analytical purposes due to its inefficient conversion of hydrogen peroxide.

In one embodiment the present invention overcomes the deficiency of H_2O_2 dependence by making use of the large chemiluminescent reservoir of energy in the oxalate system's chemistry. By using a suitable quantity of hydrogen peroxide and oxalate, a vast amount of energy may be generated in a form which is then released as chemiluminescence upon the introduction of a fluorescer.

Thus, the oxalate, acting in a fashion which can be visualized as analogous to a charged chemical battery, releases the stored energy to the fluorescer-conjugate in the same manner as an electrical switch in a circuit releases the energy of a battery to a lamp. This "switch" action causes chemiluminescence and, by incorporating the fluorescer to a detector of the analyte of interest, one can employ the reaction to trigger a detection system both qualitatively and quantitatively related to the analyte to be measured.

It is, therefore, an object of the present invention to provide for a system for the detection of a biological analyte of interest comprising an encapsulated fluorescer material which has been conjugated to an immunological specie specific to the biological analyte of interest, a means of disrupting the capsule containing the fluorescer and an energy source other than electro-magnetic radiation which is capable of activating the fluorescer.

A further object of the present invention is to provide for a qualitative method for the detection of a biological analyte of interest comprising:

- (a) labeling an immunological specie specific to the analyte of interest with an encapsulated fluorescer material which is biologically compatible with such specie;
- (b) contacting the encapsulated fluorescer labeled specie and the biological of interest to form an encapsulated fluorescer labeled specie/biological complex;

(c) separating the fluorescer labeled specie/biological complex;

(d) disrupting the capsule containing the fluorescer label thus freeing it to solution;

(e) contacting the freed fluorescer with an energy source other than electro-magnetic radiation which is capable of activating the fluorescer label; and

(f) determining the presence or absence of chemiluminescent light emitted from the activated fluorescer.

A further object of the present invention is to provide for a quantitative method for measuring the amount of a biological analyte of interest comprising:

- (a) labeling an immunological specie specific to the analyte of interest with an encapsulated fluorescer material which is biologically compatible with such specie;

(c) contacting the encapsulated fluorescer labeled specie and the biological of interest to form an encapsulated fluorescer labeled specie/biological complex;

(d) disrupting the capsule containing the fluorescer label thus freeing it to solution;

(e) contacting the freed fluorescer with an energy source other than electro-magnetic radiation which is capable of activating the fluorescer label; and

(f) determining the presence or absence of chemiluminescent light emitted from the activated fluorescer.

A further object of the present invention is to provide for a novel class of micro encapsulated fluorescer materials which may be conjugated to an immunological specie specific to a biological analyte of interest to provide a means for the detection of such biological.

A further object of the present invention is to provide for a novel class of conjugated microencapsulated fluorescer/biological compositions useful in the detection of various biological analytes of interest.

A further object of the present invention is to provide for test kits for the detection of a biological analyte of interest employing the microencapsulated fluorescer materials described herein.

DESCRIPTION OF THE INVENTION

According to the present invention, there is provided a system for the detection of a biological analyte of interest comprising an encapsulated fluorescer material which has been conjugated to an immunological specie specific to the biological analyte of interest, a means for disrupting the capsule containing the fluorescer and an energy source other than electro-magnetic radiation which is capable of activating the fluorescer.

There is also provided a method for the qualitative and/or quantitative method for the detection of a biological of interest comprising:

- (a) labeling an immunological specie specific to the analyte of interest with an encapsulated fluorescer material which is biologically compatible with such specie;

(b) contacting the encapsulated fluorescer labeled specie and the biological of interest to form an encapsulated fluorescer labeled specie/biological complex;

(c) separating the encapsulated fluorescer labeled specie/biological complex;

(d) disrupting the capsule containing the fluorescer label thus freeing it to solution;

(e) contacting the freed fluorescer with an energy source other than electro-magnetic radiation which is capable of activating the fluorescer label; and

(f) detecting the presence of and/or measuring the quantum of chemiluminescent light emitted.

Additionally, there is provided novel microencapsulated fluorescer and conjugated microencapsulated fluorescer/immunological specie compositions useful in the detection of various biological analytes of interest.

Further there is provided novel test kits for the detection of a biological analyte of interest employing the microencapsulated fluorescer materials described.

With respect to Charts I, II, and III, Rauhut et al. (1969), Chemiluminescence From Concerted Peroxide Decomposition Reactions, *Accounts of Chemical Research*, Vol. 2, 80-87, it can be seen that one mole of H_2O_2 is necessary to convert one mole of luminol into one mole of the energized or excited molecule. This excited molecule then reverts to its ground state and emits light. Of interest is the fact that the CL compound, in Chart I, luminol or its derivatives, is also capable of converting the chemical energy of the system to light. Thus, the luminol acts as a source of CL energy and also as a fluorescer to absorb the energy and produce visible light. The luminol system is, therefore,